7

**NEUROMEDIN C DECREASES A POTASSIUM CONDUCTANCE AND INCREASES A NON-SPECIFIC CONDUCTANCE IN SUPRACHIASMATIC NEURONES, IN RAT BRAIN SLICES IN VITRO.** T. Reynolds, R.D. Pinnock, G.N. Woodruff, Parke-Davis Neuroscience Research Centre, Hills Road, Cambridge, England.

The two bombesin receptor subtypes show a differential distribution in the CNS (Battey et al., 1991). The suprachiasmatic nucleus (SCN) contains a high density of GRP receptors and neuromedin C (NMC) has been shown to excite neurones in this area (Pinnock et al., 1994). In the present study we have investigated the mechanism of action of NMC using whole-cell patch clamp recording. Rat brain slices (approximately 350-400μm thickness) were prepared from male Wistar rats (50-100g) as described previously (Pinnock et al., 1994).

NMC (10nM) was shown to excite 8 out of a total of 32 neurones in current clamp. A small increase in neuronal input resistance and membrane depolarisation was seen with 100nM NMC when neurones were voltage clamped at -60mV, NMC (100nM) was shown to induce an inward current in the presence of 1μM tetrodotoxin (n = 26). I-V curves determined from these neurones suggested the existence of two neuronal subpopulations. Six cells displayed a single reversal potential (-98.5 ± 1.4mV (mean ± SE mean)) which is close to $E_K$ (-97.6mV). Whilst I-V curves from the remainder (n = 20) displayed two reversal potentials which were close to $E_K$ (-100.6 ± 3.2 mV, n = 8) and -30.2 ± 2.1mV, n = 12 suggesting a mixed ionic event.

In this latter neuronal subpopulation, the inward current at -60mV persisted in $K^+$-free external and internal solutions, where $K^+$ was replaced by the $Cs^+$ salt (n = 4). Furthermore this current was not reduced by substitution of choline or N-methyl, D-glucamine for $Na^+$ (leaving 25mM sodium) (n = 4) or addition of $Cd^{2+}$ (0.2mM) (n = 3). Removal of external calcium alone did not reduce the inward current (n = 1).

The present data suggests that NMC excites a distinct subpopulation of neurones in the SCN by decreasing a potassium conductance and increasing a non-specific cation conductance with varying contributions from each conductance in each neurone.

References: Battey, J. & Wada, E. (1991), TINS, 14(12), 524-528; Pinnock, R.D., Reynolds, T. & Woodruff, G.N. (1994), Brain Res., 653, 119-123.

8

**PROHORMONE THIOL PROTEASE (PTP) PROCESSING OF RECOMBINANT PROENKEPHALIN.** *@M.R. Schiller, +L. Mende-Mueller, #K.W. Miller, V.Y.H. Hook. Dept. of Medicine, Univ. of Calif., San Diego; *Dept. of Biochemistry, Uniformed Services University, Bethesda, MD; +Dept. of Biochemistry, Medical College of Wisconsin, Milwaukee, WI; #Dept. of Molecular Biology, Univ. of Wyoming, Laramie, WY; @present address, Dept. of Neuroscience, Johns Hopkins Univ., Baltimore, MD.

The "prohormone thiol protease" (PTP) from adrenal medullary chromaffin granules has been demonstrated as a novel cysteine protease that converts the model enkephalin precursor, $^{35}$S-(Met)-preproenkephalin, to appropriate enkephalin related peptide products. In this report, PTP processing of authentic proenkephalin (PE) was examined with respect to production of appropriate intermediate products, and kinetics of PE processing, were assessed. Recombinant PE was obtained by high level expression in E. coli, with the pET3c expression vector; PE was then purified from E. coli by DEAE-Sepharose chromatography, preparative gel electrophoresis, and reverse-phase HPLC. Authentic purified PE was confirmed by amino acid composition analyses and peptide microsequencing. In time course studies, PTP converted PE (12 mM) to intermediates of 22.5, 21.7, 12.5, and 11.0 kDa that represented $NH_2$-terminal fragments of PE, as assessed by peptide microsequencing. Differences in $M_r$'s of the 22.5, 21.7, 12.5, and 11.0 kDa products reflect PTP processing of PE within the COOH-terminal region of PE, which resembles PE processing in vivo. Products of 12.5, 11.0, and 8.5 kDa were generated by PTP cleavage between Lys-Arg at the COOH-terminus of (Met)enkephalin-Arg$^6$-Gly$^7$-Leu$^8$. The 8.5 kDa product may represent peptide I, which is present in adrenal medulla; the 12.5 and 11.0 kDa fragments most likely contain peptide I and peptide E. Kinetic studies indicated that PTP has a $K_{m(app)}$ value of 18.6 mM PE and $V_{max(app)}$ of 1.98 mmol/hr/mg. These kinetic constants are consistent with estimated intragranular levels of PE and PE-derived products. These results demonstrating PTP conversion of PE to intermediates resembling those in vivo, and kinetcis that are compatible with in vivo processing of PE, implicate a role of PTP in PE processing.